

**A Review
Of Marking Techniques
For
Amphibians And Reptiles**

by

John W. Ferner



B. P. Paschke

society for the study of amphibians and reptiles

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Preface

Since Woodbury's (1956) review of marking techniques for amphibians and reptiles over twenty years ago, many new and improved methods have been published. More field and behavioral studies which require marking are now being conducted than in the past and often investigators are in need of marking techniques with very specific qualifications. It is for these reasons that I am writing this review of marking techniques for amphibians and reptiles.

Some criteria for an ideal mark or tag are as follows:

1. It should not affect the survivorship or behavior of the organism (Ricker, 1956).
2. It should allow the animal to be as free from stress or pain as possible (Lewke and Stroud, 1974).
3. It should identify the animal as a particular individual, if desirable.
4. It should last indefinitely (Ricker, 1956).
5. It should be easily read and/or observable (Lewke and Stroud, 1974).
6. It should be adaptable to organisms of different sizes (Lewke and Stroud, 1974).
7. It should be easy to use in both laboratory and field, and use easily obtained material at minimal cost (Lewke and Stroud, 1974).

There are, of course, no techniques that satisfy all of these desired criteria to the fullest. Selection of a technique, then, will require deciding which of the criteria are most important for any particular study. Often, two or more marks are used in order to meet more of the criteria (e.g., toe clipping and dorsal paint symbols). Also, a technique described for use with one group of herpetofauna might be adapted for use with a new group and therefore one might benefit by scanning other portions of this guide. There is a need for more standardization of marking techniques in comparative studies or those that might be carried on by other investigators in future years.

In order to have some measure of the techniques most commonly used at present, I surveyed all the herpetological articles printed during the past seven years in five journals (*Copeia*, *Ecology*, *American Midland Naturalist*, *Herpetologica* and *Journal of Herpetology*). This information has been used in this review to give some indication of what techniques are most acceptable and in use today. I have given most space to the more commonly used techniques and to the newer ones which may not have yet stood the test of time. When available, information on the advantages and disadvantages of each technique has been given.

For the most part, this guide attempts to give information adequate for the use of techniques without consulting the original source. However, once a technique has been tentatively selected it seems advisable to consult the original source if

your time and facilities permit. The use of very complex techniques, such as those using radioisotopes or radiotelemetry, require careful review of the original sources. Those involved techniques usually need to be adapted for each particular study. Radioisotopes and radiotelemetry are covered in separate chapters because of their general appeal and the many aspects of their use which are applicable to a variety of animals. Discussion of marking techniques for the more exotic reptiles, such as sea turtles and crocodilians, is limited due to the relatively few investigators working with these organisms. An additional shortcoming of this review is that many valuable techniques have no doubt been omitted, primarily due to my not having uncovered them in the literature.

I would like to thank Clarence J. McCoy, Richard G. Zweifel, Roger W. Barbour and Michael V. Plummer for assistance in obtaining references. Ray E. Ashton, Jr. provided a significant amount of information concerning the use of radioisotopes. Helpful comments on the use of radiotelemetry and radioisotopes with a variety of reptiles were given by Henry S. Fitch. Unpublished information on the use of lasers and freeze branding as marking techniques was made available by R. Keith Farrell. Thanks are also extended to Stephen J. Gorzula for information on the scale clipping of caimans. William S. Bryant provided valuable suggestions in reviewing the manuscript. Karen Brauer redrew all figures from originals.

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Larval Amphibians

Due to their small size and delicate tissues, amphibian larvae require special marking techniques. A traditional method, used by Turner (1960), simply involves clipping notches from the tail fin of tadpoles. Guttman and Creasey (1973), however, reported that the fin clipping technique causes a higher mortality than staining tadpoles with Neutral Red. Herreid and Kinney (1966) first used this staining technique by placing tadpoles of *Rana sylvatica* in shallow pans with a 0.05% pond water solution of Neutral Red for 30 minutes. In laboratory studies they found that dyed and undyed tadpoles survived equally well. Herreid and Kinney (1966) found this staining technique adequate for a mark-recapture study, although the dye usually faded within one week. Neutral Red has also been used successfully to stain tadpoles of *Rana clamitans* which were immersed for 3 hours in concentrations of 1 part stain to 25,000 or 50,000 parts of pond water (Guttman and Creasey, 1973). These tadpoles developed a bright crimson venter, had an immediate mortality rate of 8.7% (N=567) and retained the stain for at least 10 days. All staining methods appear to be time limited.

A more permanent marking method has been described for *Rana* and *Ambystoma* larvae by Seale and Boraas (1974). This technique uses a subcutaneous injection of an organic dye-organic solvent mixture into the tail fin. The authors, by trial and error, determined the ideal ratio of mineral oil to petroleum jelly of 21:20 (by weight). If an excess of either component is used, undulations of the tail fin may cause the ejection of the marks. The ratio may vary depending on the viscosity of the commercial products used, but once determined, the same ratio can be used for all species. Organic biological stains ("Oil Red O" and "Oil Blue M" from Matheson, Coleman and Bell) were used to dye the oil. Variations in color and placement of marks were used to identify groups of individuals marked on specific dates. Marks were placed in the dorsal or ventral tail fin cavity adjacent and parallel to tail musculature by using a 22-gauge hypodermic syringe. The needle was inserted about 0.6 cm into the cavity and then removed while pressure was applied with the syringe plunger. This procedure left a 0.5 cm line of dye. No mortality, infection, impairment of mobility or retardation of growth were found as a result of this marking technique. Any individuals injured during the marking process were removed before release and caution was particularly needed to prevent injury to the gills of larval *Ambystoma*. During metamorphosis, this organic dye-organic solvent mark is resorbed with the tail with no discernible ill effects.

An additional method of marking salamander larvae, using fine grained (20 μ) fluorescent pigments, was used by Ireland (1973). Four colors of these pigments (solid solutions of fluorescent dyes in a melamine-sulfonamide-formaldehyde resin) were mixed with acetone to make a paste. This paste was then administered to the larvae on their mid-dorsal surface with a blunt probe which had been heated with a propane torch. The probe burned through the outer epithelial layers, leaving a small scar (1 mm). The epithelium regenerated within 15 days and incorporated the fluorescent pigments. These

tags were then observed by placing the larvae in a petri dish containing water, placing the dish in a black box and then exposing them to long wave ultraviolet light. In the laboratory, 50% of the *Eurycea multiplicata* tested had lost their fluorescent tag in 70 days and 47% of the *Ambystoma annulatum* had done so. It appeared as though loss of the tags in the field was less than this. No detrimental effects of this tag on the larvae were observed.

In summary, studies of a particular species may find one of the above techniques more applicable than the others. As these new techniques are used with additional species in the future, more information on their relative merits will be available. However, the technique of Seale and Boraas (1974) appears to be the most permanent and least detrimental to amphibian larvae. On the other hand, the staining method of Guttman and Creasey (1973) seems to be a much simpler technique to master. The detrimental effects, complexity, and time limited nature of the other techniques discussed discourages their use.

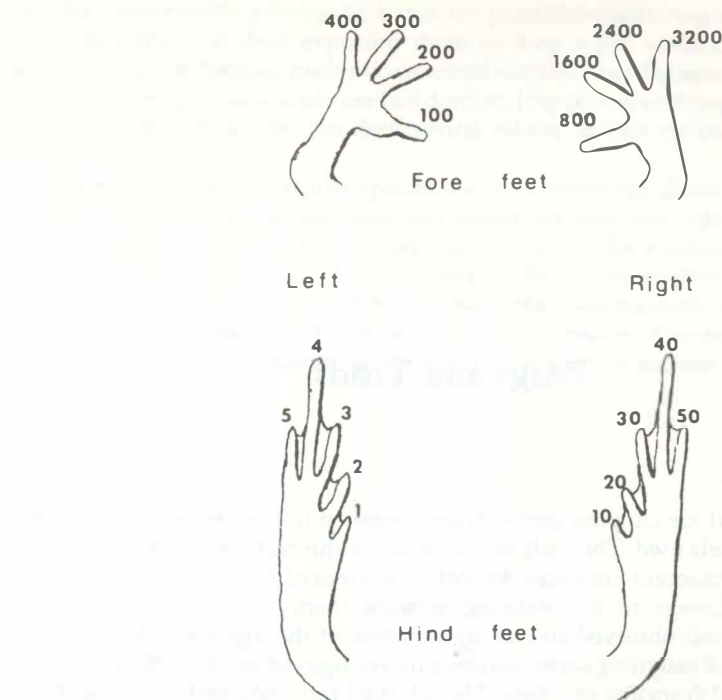
Frogs and Toads

TOE CLIPPING

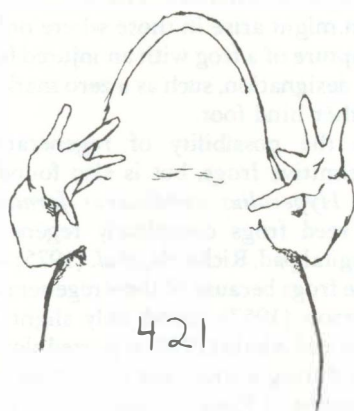
Although several toe clipping methods are proposed in the literature, that of Martof (1953) is most widely used. The early modification of this technique by Carpenter (1954) has not been cited in recent literature. Martof cut the toes of *Rana clamitans* with scissors, trying to avoid damage to the webbing between them. Little blood loss or loss of swimming power was observed and no regeneration of the digits was found. Figure 1A shows the system of assigning serial numbers to the digits of the feet. No more than two toes were removed from any one foot. The left hind foot indicated units, and for those larger than five, combinations of two digits (the fifth and one other) were excised. For example, clipping the fifth and third toes designated tens and the front feet denoted hundreds. With this arrangement, one can mark 6399 individuals in series; this highest number can be indicated by removing the two outer digits on each foot. The only concern Martof expressed about this plan was that confusion might arise in those where only a single toe on a single hind foot was removed with capture of a frog with an injured foot. He suggested alleviating this problem with a special designation, such as a zero marking by removal of the second and fourth toes on the other hind foot.

One potential problem with toe clipping is the possibility of regeneration. Regeneration may be more commonly found in primitive frogs, but is also found in advanced forms such as the Kenyan reed frog, *Hyperolius viridiflavus ferniquei* (Richards, *et al.*, 1975); newly metamorphosed reed frogs completely regenerate amputated toes, including the structurally complex digital pad. Richards, *et al.* (1975) also noted that ecologists generally avoid toe clipping tree frogs because of their regenerative capabilities. This depends on the species, as Jameson (1957) found only slight toe regeneration in *Hyla regilla* after one year, and Brown and Alcala (1970) reported slow or nonexistent regeneration of toes in *Rana erythraea* during a four year field study.

Briggs and Storm (1970) avoided clipping the thumbs of *Rana cascadae* due to their importance in amplexus and usefulness in sexing individuals. This modification was also made by Dole and Durant (1974) who additionally did not clip the smallest toes in their study of *Atelopus oxyrhynchus*. The most serious criticism of toe clipping anurans was raised by Clarke (1972) who reported that the probability of recapture of *Bufo woodhousei fowleri* decreases as the number of digits excised increases. His technique involved the removal of 1 or 2 toes per foot, complete removal on the front feet, but only to the webbing in back. Another report by Daugherty (1976) indicates a problem with weight loss in *Rana pipiens* which were toe clipped. The implications of these reports are far reaching for the use of toe clipping with all amphibians and reptiles. Even considering these disadvantages, toe clipping is still the most common marking technique used for anurans.



A



B



C

Figure 1. A. Drawing from Martof (1953) illustrating the numbering system for toe clipping anurans. See text for details.
 B. Kaplan's (1958) illustration of numerals on the venter of an anuran which incorporate India ink into scarified tissue.
 C. A numbered bird band used to tag an anuran on a toe (redrawn from Kaplan, 1958).

BRANDING

Kaplan (1958) described a branding technique for frogs which involves the incorporation of India ink into scarified skin of the venter (Fig. 1B). Numeralized grooves are etched in the skin with a hypodermic needle and then filled with India ink. Erythema disappeared quickly, no infection was observed and the numerals remained distinguishable for over three months. Kaplan (1958) reported on an electric tattooing technique as an improvement upon the scarification method. He used a small electric tattoo marker with a narrow needle to write fine numerals or letters on the ventral surface of frogs. Higgins India ink was found most effective and was mixed with a drop of glycerin to aid its spreading into the skin. Surplus ink was wiped away, leaving a clear, permanent mark. Some initial local inflammation was observed in the frogs, but was only rarely prolonged.

Heated branding irons have been used to mark toads and frogs (Clark, 1971). The irons were shaped into numerals from a wire which was 20% chromium and 80% nickel, B and S gauge No. 24 (Hoskins Chromel "A" resistance wire made by The Malin Co., Cleveland, Ohio). This wire proved best because it can be heated red-hot repeatedly without oxidizing appreciably. The brands were heated with a propane torch or Bunsen burner and applied to the ventral side long enough to penetrate the dermal layer. Clark reported that recaptured individuals of *Bufo valliceps* "showed a glossy, brown, horny layer over the area of each numeral." In most toads, this layer disappeared within two weeks. Legible brands were recorded on toads for up to 21½ months. This technique was also used successfully with *Rana catesbeiana* and *R. pipiens*; data from these species suggested that the branding method is similar to toe clipping in its effects on survival.

Branding of anurans has also been accomplished with the use of silver nitrate (Thomas, 1975). Seven adult *Hyla cinerea* were branded using commercial silver nitrate applicators (75% silver nitrate and 25% potassium nitrate) of the type often used for veterinary cauterization. Narrow lines were traced from one to three times on the back of the frog causing a brown mark to form immediately. One individual accidentally had the mark smeared over most of its back and died within five days. The other tree frogs were apparently not adversely affected by the marks. Within about two weeks, the dark mark fades into a light mark which also contrasted well with the background color of the frogs. Thomas recommends this method for dark colored amphibians and found the marks to remain distinguishable for over 9 weeks. The silver nitrate applicator needs to be moistened frequently when marking dry-skinned amphibians.

The silver nitrate technique seems inappropriate for marking large numbers of anurans for individual recognition. It does, however, appear to be a good alternative to some other methods in that the mark is on the dorsal surface and recapture may not be necessary for identification.

Daugherty (1976) has freeze branded *Ascaphus truei* with branding irons fashioned from No. 12 A.W.G. insulated copper electrical wire. The numerals which were shaped at the insulation-stripped end of the 12 cm irons were cooled in chipped dry ice for a minimum of 30 minutes prior to initial use; continued use required only about 30 to 60 seconds of reimmersion. Brands were made on the ventral surfaces of the frogs for about 10 seconds and there was no penetration of the integument by overapplication. The freeze brands could be easily read within one day and brands were observed to last more than two years in the field. The mark gradually lost pigment, so that after one year the viscera were often visible through the integument. There was some indication (from one frog) that brands may not remain visible in individuals marked just after metamorphosis. All other recaptured adults and juveniles (N=98) had clearly visible brands remaining. The author indicates that this technique has a minimal impact on an anuran's life history in that the brand is inconspicuous and produces "no continuing energy drain on an individual." An important consideration in the field use of freeze branding is the availability and cost of dry ice.

TAGGING

Woodbury's (1956) review of the jaw tagging technique of Raney (1940) did not include the report by Stille (1950) that the loss of these tags by toads was significant. Woodbury (1956) did cite a personal communication with Stebbins which stated that the jaw tags were often lost and "caused considerable irritation." This technique is no longer used.

Kaplan (1958) used aluminum toe bands to tag frogs (butt-end bird band, # 1242, size 2½, National Band and Tag Co., Newport, Kentucky 41071). These numbered, cylindrical bands were placed around a toe (see Fig. 1C) and the two ends were pressed together with pliers. The bands were tightened so as not to restrict circulation, but they pierced the webbing of the foot. These tags remained fixed indefinitely and caused no apparent reduction in the movement of the frog.

A nylon waistband was used by Emlen (1968) to aid in the behavioral study of male *Rana catesbeiana*. This tag was chosen because it allowed individual identification in the field without disturbing the bullfrog. Waistbands were made from 13 mm wide, preshrunk, nylon elasticized banding. These were about 13 cm long and painted with distinctive, colored patterns or black numerals using Testor's butyrate dope paint. Each strip was then stapled into a circular band which was looped over the bullfrog's hind legs and positioned around the pelvic girdle. The bands fit snugly so as to prevent rotation or catching on items in the environment, but loosely enough to permit normal activity and respiration. Color-marked waistbands were recognizable from maximum distances of about 8 to 12 m using a headlamp and binoculars, while numbered bands were only visible from 4 to 6 m. Emlen reported no differences in behavior, mortality, emigration rates, or weight loss between tagged and untagged frogs. Due to soiling and staining, seasonal replacement of these waistbands would be necessary.

TRAILING DEVICE

A trailing device using nylon sewing thread was devised by Dole (1965) to study the movements of individual *Rana pipiens*. A sewing machine bobbin was placed in a holder constructed from a piece of rigid plastic tubing with a flat plastic base glued to one side and then attached on a 6.3 mm wide, elastic band. The bobbin was held in place with a wire passing through two holes in the top of the holder. The thread trailed out through a slot cut in the back of the holder and the entire device was secured around the waist of the frog. A small stake was used to mark the point of capture and the trailing thread was tied to it. The 50 m of thread on the bobbin lasted from one hour to seven days, depending on the activity of an individual. Empty bobbins were easily replaced in the field without removing the elastic saddle. The loaded trailing device weighed about 8.5 g and "did not seem to hinder the animal's jumping ability seriously." The device may have shortened their jumping length and no individuals less than 60 mm long were trailed. Dole reported some difficulty with frogs swimming and entering crevices with the device. If waistband irritation occurred on the skin, as it occasionally did, the device was removed immediately until complete healing took place. Grubb (1970) modified Dole's technique by using 200 m of cotton thread.

Salamanders

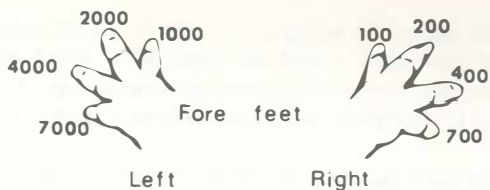
MUTILATION TECHNIQUES

At the time of Woodbury's (1956) review, toe clipping was the only successful marking technique reported for salamanders and was just beginning to come into use. Toe clipping has been the most common means of marking salamanders in recent years and in most cases something similar to Martof's (1953) system of marking anurans is used. The system used by Twitty (1966) in marking *Taricha* is one example (Fig. 2).

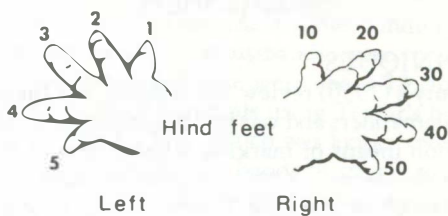
Table 1 summarizes some reports in the literature concerning the regeneration of digits in salamanders. It appears, then, that regeneration needs to be considered, but whether or not it is a deterrent to using toe clipping depends upon the species. Heatwole (1961) controlled regeneration of digits in *Plethodon cinereus* with a beryllium nitrate treatment. He found concentrations of beryllium nitrate 0.1 N or greater completely inhibited regeneration (N=6) and recommended using the lowest possible dosage to avoid any potential harmful effect. Heatwole simply dipped the limb in the beryllium nitrate solution for 1.5 minutes and then rinsed it with distilled water. Beryllium nitrate should be used only with extreme caution in that it is toxic to man and can easily diffuse through the skin. Extreme care, rubber gloves and forceps are important precautions if using beryllium nitrate. Efford and Mathias (1969) reported that neither food consumption nor exposure to beryllium nitrate solution affected regeneration in *Taricha granulosa*. They did discover, however, that temperatures below 10°C inhibited regeneration of digits, which would be an important factor in field studies. At 18°C, *T. granulosa* had a significant amount of toe regeneration.

Healy (1974) marked post-larval metamorphs of *Notophthalmus v. viridescens* by "amputating one limb at the middle of the zeugopodium." He questioned the influence of this on the mortality rate because relatively few individuals were recaptured. Juvenile *Desmognathus f. fuscus* have toes too small for precise clipping, but have been successfully marked by clipping small pieces of the tail at either right angles to the longitudinal axis of the body or in the transverse plane at different angles (Orser and Shure, 1972). This technique allowed groups of marked juveniles to be distinguished for at least one month before regeneration became a problem.

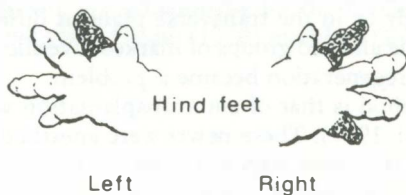
A unique mutilation method is that of autotransplantation which has been used for *Triturus alpestris* (Raginski, 1977). These newts were anesthetized with 0.1% MS 222 and 3 mm square pieces of skin were removed from the orange venter and the bluish to brown-black dorsum using fine forceps and scissors. These grafts were exchanged and needed no adhesives. After transplantation, which took about 3 minutes, the newts were kept isolated out of the water for an hour. Individual recognition was possible by using various combinations of the locations and number of grafts. Raginski reported a 95% success rate and retention of the grafts for at least 3 years. This technique may prove very useful for long term marking of many salamanders, but is more difficult to use with species having spotted patterns and cannot be used with larvae.



A



B



4372

Figure 2. The numbering system used by Twitty (1966) in toe clipping salamanders.
 A. The numerals assigned to each digit.
 B. In order to obtain an individual numbered 4372, the shaded toes would be clipped.

TABLE I

Digit Regeneration Times for Selected Toe clipped Salamander Species.

Species	Time Interval for Regeneration	Source
<i>Taricha</i> spp.	Several years	Twitty (1966)
<i>Taricha granulosa</i>	Indefinitely, when kept below or at 10° C	Efford and Mathias (1969)
<i>Plethodon cinereus</i>	7 months in laboratory	Heatwole (1961)
<i>Plethodon glutinosus</i>	At least two years	Wells and Wells (1976)
<i>Plethodon wehrlei</i>	50% after 100 days; regenerated digits identifiable by lack of pigmentation	Hall and Stafford (1972)
<i>Cryptobranchus alleganiensis</i>	One year	Hillis and Bellis (1971)
<i>Batrachoseps</i> spp.	Slow and regenerated toes recognizable	Hendrickson (1954)

BRANDING

Taber, *et al.* (1975) branded *Cryptobranchus alleganiensis* with heated, 1.5 cm high, numeral brands of thin nichrome wire. In most cases, these brands remained clear through a two year study, but some did fade and required rebranding. This technique is similar to that of Clarke (1971) which could probably be modified for caudates. The freeze branding technique of Daugherty (1976) was not used with salamanders, but also seems applicable. Woolley (1962) used a black, Carter's felt ink pencil to mark salamanders. Dilute acetic acid or ammonium hydroxide was used to remove slime on the salamander's tail before application. These marks lasted at least a month.

TAGGING

Floy T-tags were used successfully to mark *Cryptobranchus alleganiensis bishopi* by Nickerson and May (1973), but no details of the procedure were discussed. Whiteford and Massey (1970) tagged *Ambystoma tigrinum* by suturing a numbered, plastic float through the tail with a monofilament line. The line was made long enough to allow these salamanders to move through the deepest portion of the lake.

Woolley (1973) tagged *Eurycea lucifuga* and *E. longicauda melanopleura* with a subcutaneous injection of two parts Liquitex Acrylic Polymer to one part distilled water. This mixture was injected into the lateral proximal caudal region with a 22 gauge hypodermic needle, leaving a mark 7 to 10 mm in diameter. Woolley observed no adverse effects with this procedure and found slight fading in only a very few individuals. Although this study did not require the recognition of individuals, the author suggests that a series of acrylic colors could be used to differentiate individuals. Woolley found this to be a convenient, relatively harmless technique which allowed quick recognition in the field with a reduction in subsequent handling.

INTEGUMENT PATTERN

Perhaps the most ideal method of recognizing individuals is to use their own, naturally occurring variation in morphology. This has been done for *Triturus cristatus* and *T. vulgaris* by photographing belly patterns (Hagstrom, 1973). In using this technique, one must be sure that the patterns are both recognizably different and constant through time. Healy (1975) found the variation in the dorsal spot pattern of *Notophthalmus viridescens* useful in identifying individuals. This technique requires recapture and handling which may not be consistent with the animals' behavior.

Turtles

SHELL NOTCHING

The most commonly used marking technique for turtles is that of notching the shell. Cagle (1939) developed a coding system for notching which has had wide usage (see Fig. 3). Marginal plates are numbered 1 to 11 on each side of kinosternid turtles and 1 to 12 on chelydrid, emydid and testudinid turtles. In addition, Cagle suggested notching the plastron to give a number identification to each carapace series and therefore increase the potential number of turtles that could be marked. By using four marginal notches, 2516 marking combinations could be made. Marks were recorded by having a comma separating those made on one side of the carapace, and a hyphen to separate the left and right sides. Therefore, a turtle designated 2, 6-3, 5 had the second and sixth marginals on the left side notched and the third and fifth on the right. Cagle used a triangular file to make the notch and a square-edge file to widen it to one-third to one-half the width of the plate. Young individuals with unossified carapaces were marked using a small pair of sharp scissors. Ernst (1971) used a hacksaw blade to notch adults of *Chrysemys picta* and marked juveniles with a sharp pen knife.

Ernst, *et al.* (1974) evaluated the notching technique as being best for marking great numbers of turtles in mark-recapture studies because of low cost and the need for few tools. However, they also proposed a new coding system (Fig. 3) which will allow the consecutive numbering of turtles as can be done with some numbering systems in the toe clipping of lizards and amphibians. A number for each individual is obtained by adding the numerical values of the marginals which have been notched. By using plastron marks on the gulars and anals, an almost unlimited number of combinations can be used. This system can be abbreviated for use with kinosternid turtles which have fewer scutes. The authors suggest that marginals at the bridge or junction of the plastron and carapace should not be notched so as not to weaken the shell.

Cagle (1939) cautioned that these marks may not be permanent when used in young specimens whose shells are not yet completely ossified. Therefore, he also marked juveniles by clipping the first phalanx of toes with a numbering system similar to that used for lizards.

TAGGING

Kaplan (1958) tagged turtles with numbered, aluminum bands (National Band and Tag Co., Newport, Kentucky 41071) fastened through holes drilled in the carapace (Fig. 4). *Chelydra serpentina* have been tagged with aluminum plates (4.2 x 1.6 cm) which were attached with .020 gauge, stainless steel trolling line through two 3 mm holes drilled through the marginal scutes just to the side of the tail (Loncke and Obbard, 1977). A letter and a number were recessed into each tag and painted black to make them more visible. Loncke and Obbard evaluated their technique as being "quick, uncomplicated and successful for at least three years." They preferred it to Kaplan's method because the tag

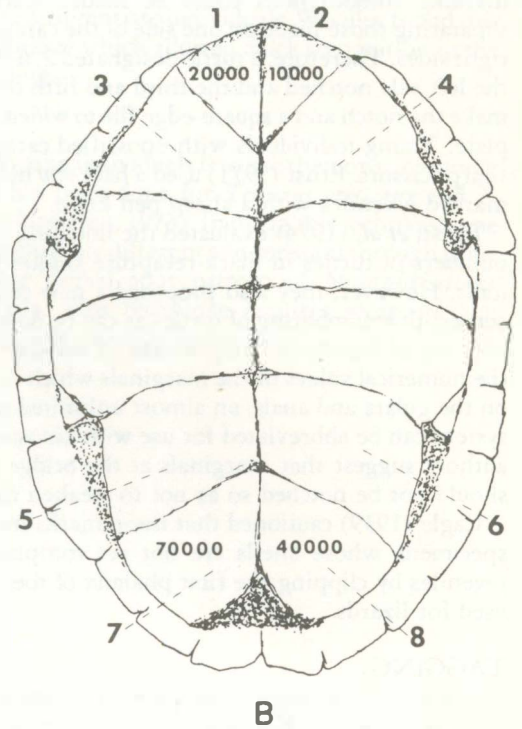
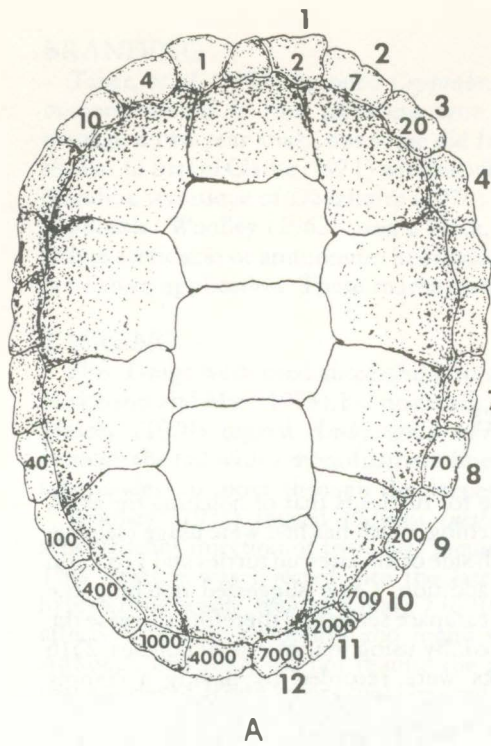


Figure 3. Numbering systems for shell notching. Cagle (1939) numbered the marginal plates on each side from 1 to 12 on the carapace (A). Plastral plates were numbered 1 through 8 (B). Ernst, *et al.* (1974) have numbered 6 plates at the cranial end of the carapace and 10 plates at the caudal end (A). In addition, they numbered four plastral scutes (B) for marking an extremely large series of turtles.

could be read at a distance, reducing the need to handle marked turtles. The turtles may only need to be handled "every few years to adjust tags and allow for growth" (Loncke and Obbard, 1977).

Turtles have also been successfully tagged by using a Buttoneer (Dennison Manufacturing Co., Framingham, Massachusetts 01701), a small tool used to fasten buttons to clothing by means of a plastic plug (Pough, 1970). The plug comes in different sizes and has a stem with a bulb at one end and a crossbar at the other. Pough (1970) placed the plug into a 3/32-inch hole drilled through a marginal plate. He suggests that this technique is good for young turtles, where regeneration has been a problem with notching. Froese and Burghardt (1975) used the plastic buttoneers to mark *Chelydra serpentina*, using a numbering system for the posterior marginal scutes. They found detachment a problem when the plugs were too short to allow free movement in the holes. However, even if the plugs were lost, the 2 mm holes were still visible.

Testudo gigantea have been marked with numbered, titanium disks which were fixed in depressions drilled into the keratin shield (Gaymer, 1973). The tags were held in the depression by a metal-resin adhesive and had a very high retention rate. Ward, *et al.* (1976) marked the carapace of *Clemmys guttata* with an "adhesive tag" which bore an identification number.

Davis and Sartor (1975) tagged turtles (*Chelydra serpentina*) by putting a 1/8 inch diameter, wooden dowel through a hole which was drilled in the nuchal scute. The dowel was held with epoxy glue and was marked at the top with colored paint or plastic tape for individual recognition. The aquatic movements of these turtles could be easily observed when they were in shallow water or near the surface. To reduce the hinderance this technique caused turtles moving in thick vegetation, two shorter dowels were connected with a piece of rubber tubing.

Sea turtles are usually marked with numbered, monel tags which are attached to the trailing edge of a fore flipper (for example, *Caretta caretta*: LeBuff and Beatty, 1971; *Chelonia mydas*: Pritchard, 1976; *Dermochelys coriacea*: Bacon, 1973; and *Lepidochelys olivacea*: Pritchard, 1976). Pritchard (1976) reported significant tag loss through both metal corrosion and tissue necrosis, particularly with *Dermochelys coriacea*. Carr, *et al.* (1974) tagged *Chelonia mydas* for visual tracking with fiberglass-coated styrofoam floats. These floats were "lens-shaped" (30 cm in diameter by 20 cm deep) and were attached on 24 m lines. A 3 volt flashlight bulb was attached to each float and powered by batteries imbedded in the styrofoam. A fiberglass mast was also attached and was topped by an orange pennant. No adverse affects were found for this flotation device.

BRANDING AND PAINTING

Woodbury and Hardy (1948) used a white-hot wire to brand the carapace plates of *Gopherus agassizii*. The scutes were all assigned numbers or letters in order to obtain individual recognition codes. If scutes were burned too deeply, complete regeneration took place, and if burned too lightly, the scar would wear off in a few years. Woodbury (1956) added that using a 12 or 14 gauge wire in a quick burn yielded the best results. Clark (1971) branded the plastron of one *Pseudemys scripta* into the underlying bone and found no evidence of regeneration or infection during the 36 days the animal was observed. According to Lewke and Stroud (1974), freeze branding has been successfully used on *Chelonia mydas*, but no details were given. R. K. Farrell (personal communication) reported unsuccessful use of a laser in marking a turtle.

Woodbury and Hardy (1948) also used a variety of colors of paint to mark carapace scutes of *Gopherus agassizii*. However, they found this to be less permanent than branding. Medica, *et al.* (1975), studying the same species, painted the last vertebral scutes and then repainted them with a different color each year. In addition to the use of radioactive tags, Bennett, *et al.* (1970) painted numbers on the carapace of *Kinosternon subrubrum*, *Deirochelys reticularia* and *Pseudemys scripta*. Bayless (1975) painted

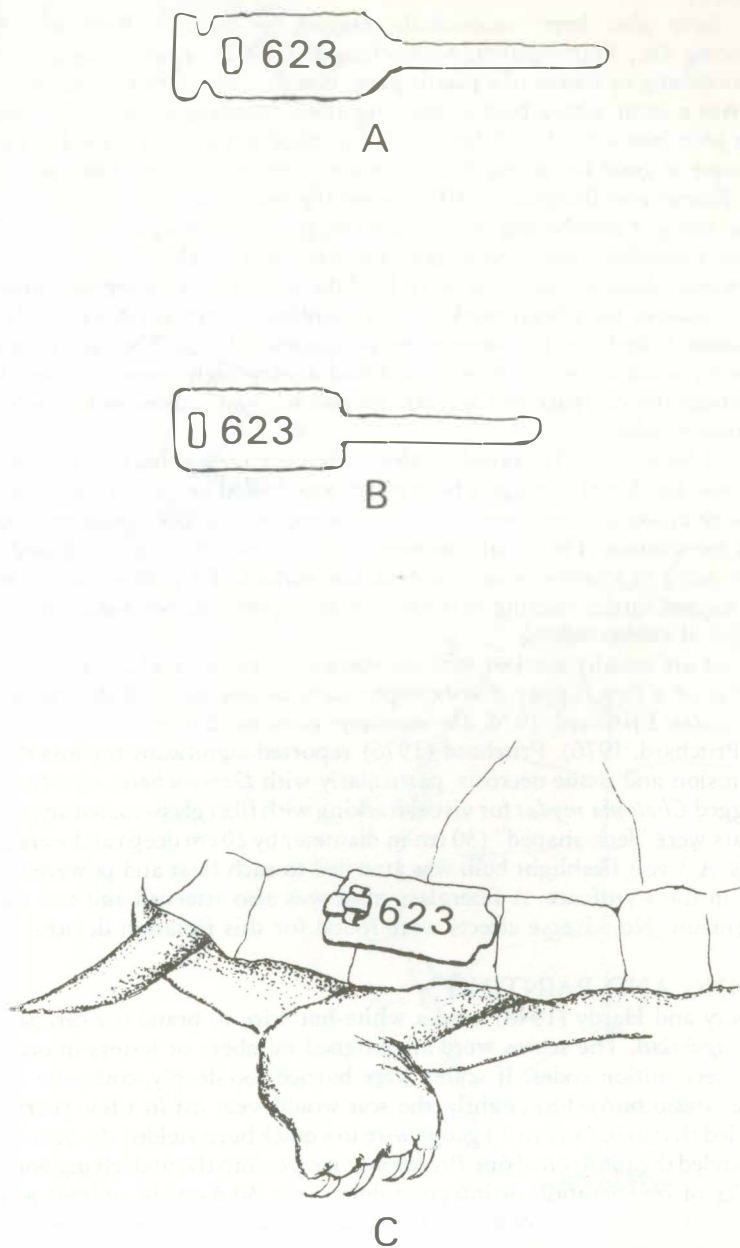


Figure 4. A. The band as it was purchased by Kaplan (1958).
 B. The band as it was modified to obtain a long tab.
 C. The band as it appeared when fastened through two holes in the carapace of the turtle.

numbers on the carapace of *Chrysemys picta* which needed to be repainted each year because they were shed with the carapace scutes. Numbers painted on the carapace allow turtles to be identified without recapture and with little disturbance.

Burger and Montevecchi (1975) marked the plastrons of nesting *Malaclemys terrapin* with washable ink so that they would not be recounted in a census while they were on land. In a study of nesting success, the eggs were numbered with a permanent ink felt pen (Burger, 1976).

TRAILING DEVICES

Although the trailing device used by Stickel (1950) for *Terrapene carolina* was not the first, it did have tremendous improvements over earlier methods. She first made a metal housing from a six ounce can (85 x 62 mm) which fit smoothly over the caudal end of the carapace, being neither wider nor higher than the shell itself. A spindle was fashioned from an iron bolt and suspended by two wire hooks which were soldered to the housing. The core of a wooden spool was reduced in size so that it would then hold about 550 yards of No. 80 white thread. The spool was mounted on the spindle and the thread strung through a wire loop soldered at the rear of the housing. The entire device was attached to the carapace with waterproof adhesive tape. Spools were wound mechanically with an adapted electric mixer and could be changed relatively easily in the field. The tape needed to be replaced occasionally due to weathering. Stickel found no evidence that this device changed the behavior of the turtles.

Lemkau (1970) attached a "thread trailer-radio transmitter packet," weighing about 70 g, to the carapace of *Terrapene carolina*. Another modification of Stickel's (1950) technique was developed by Reagan (1974) in order to avoid any interference with mating. He used 35 mm film canisters to hold a custom lathed wooden spool on which was wound 275 m of No. 50 nylon thread. The end of the thread was passed through a hole in the canister and the unit was attached to the caudal end of the carapace with waterproof adhesive tape. The end of the string was tied to an object at the release site to begin the trail. Reagan also found this method more advantageous than the old technique in that it was tangleproof, weatherproof and only weighed 12 g as compared to 55 g.

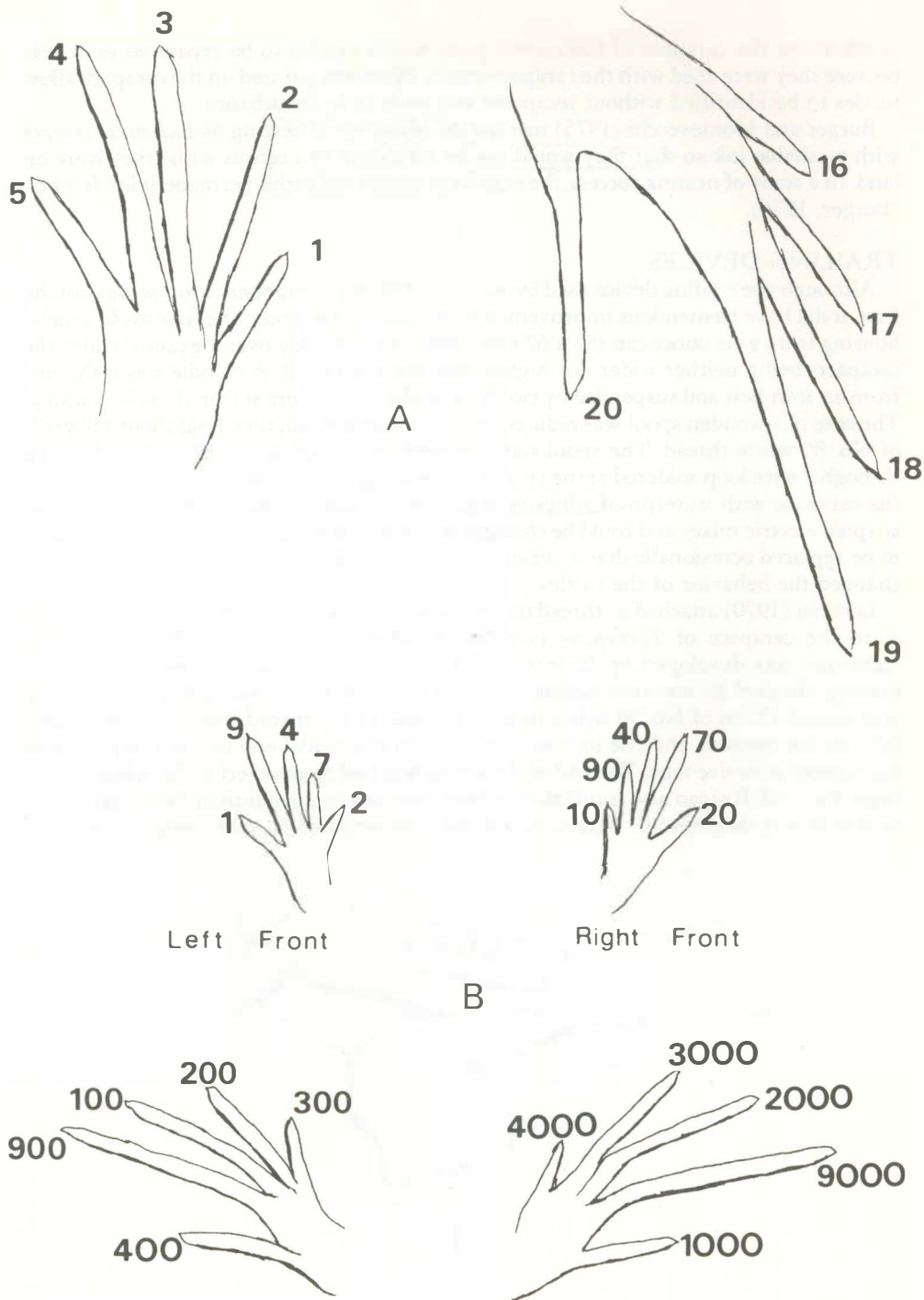


Figure 5. A. Toe numbers for the left hand and right foot as taken from Tinkle (1967). The right hand of the lizard would bear numbers 6 through 10 from the inside out and the left foot would have toes numbered 11 through 15 from the outside in.

B. The toe numbering system for lizards as taken from Medica, *et al.* (1971).

Lizards

TOE CLIPPING

Toe clipping is the most popular technique used in marking lizards. The method used by Tinkle (1967) for *Uta stansburiana* is the most commonly cited and involves clipping up to four toes, but no more than two per foot and never adjacent ones. Fingernail clippers are often used to remove the digits. The numbering system is illustrated in Fig. 5A and, for example, the removal of toes 4, 8 and 20 would give the lizard a code number 4-8-20. Medica, *et al.* (1971) described a different numbering system (see Fig. 5B) which is similar to that used for salamanders and frogs. This technique cuts at least one toe from each foot to eliminate the problem of a lizard having lost a digit(s) accidentally being mistaken for a marked animal.

Woodbury (1956) suggested lettering the feet, numbering the toes and also giving the sex for an individual an identification code. A1 D2♂ would indicate that the first toe of the left front foot and the second toe of the right hind foot of this male were removed. Many studies use a more visible mark, such as the paint symbols discussed below, in addition to toe clipping in order to reduce the need for frequent recapture. The possibility of toe clipping having a harmful affect on lizards was raised by Woodbury (1956), but none of the extensive studies of recent years have documented this as a significant problem.

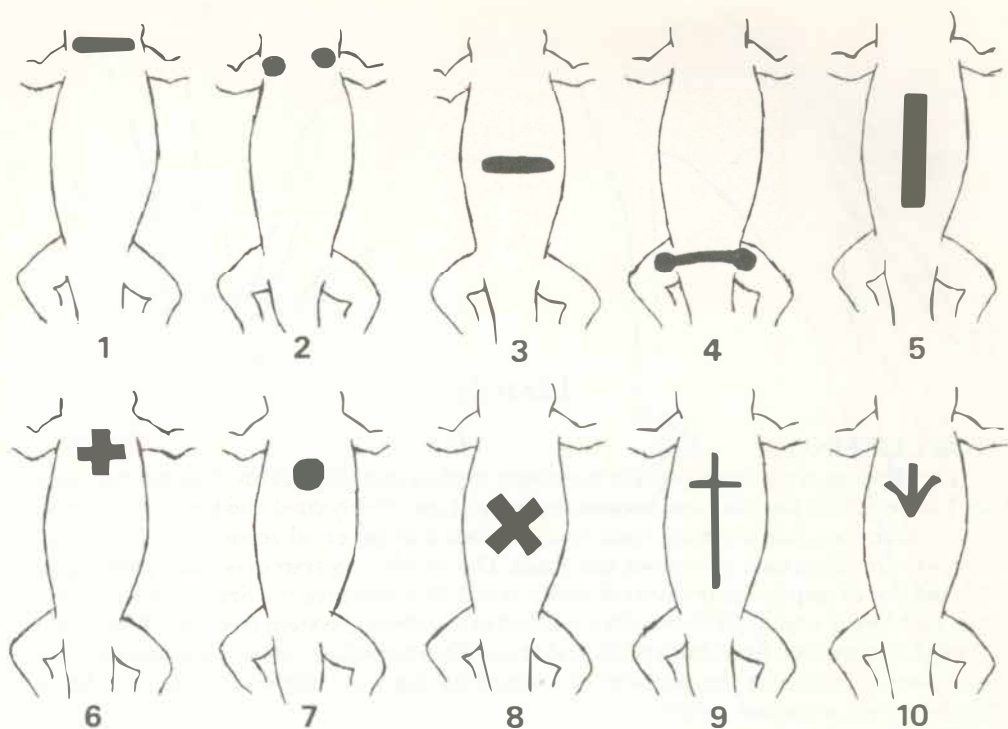
INTEGUMENT PATTERNS

In a field study with a small number of individuals of *Anolis aeneus*, Stamps (1973) found she could recognize individuals by their distinctive patterns coupled with tail regenerations in various stages. Carlstrom and Edelstam (1946) reported the successful use of black and white photographs to record unique individual dorsal patterns in *Lacerta vivipara* and throat patterns in *Anguis fragilis*.

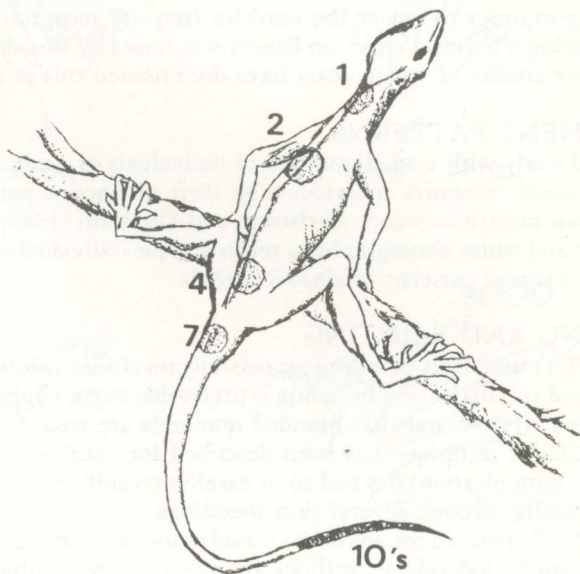
BRANDING AND PAINTING

Clark (1971) used heat branding successfully on *Anolis carolinensis* and *Phrynosoma cornutum* and speculates that branding is preferable to toe clipping in that locomotion is probably less affected and that branded numerals are easier to read than are toe clip formulae. Clark's technique has been described for anurans on page . R. K. Farrell (personal communication) has had some excellent results in freeze marking iguanas with marks persisting through several skin sheddings.

Tinkle (1967) painted the adult *Uta stansburiana* with colored insignia (see Fig. 6A) in order to identify individuals without recapture. Some confusion was caused by the shedding of skin and lizards were periodically recaptured and repainted. Young individuals were marked only with a small spot of paint on the back between the hind legs which did not allow individual recognition. Medica, *et al.* (1971) used Testor's model paint (blue, yellow, light green, pink and white) to mark lizards. They put the paint in nail polish bottles and used short (¼ inch) thinned out brushes. The symbols used by Medica,



A



B

Figure 6. A. Paint symbols used by Tinkle (1967) in various combinations for individual recognition.
 B. The paint spotting system developed by Jenssen (1970); see text for details.

et al. included such things as neck bands, longitudinal stripes, transverse bands at midbody, arrows, plus signs and dots. Jenssen (1970) painted spots with quick drying paint on the backs of *Anolis nebulosus* (Fig. 6B). Females were spotted with yellow paint and males with orange. By using combinations of the four dorsal numbers (1, 2, 4 and 7), all numbers between 1 and 9 were obtainable. The tens column was designated by painting the tails different colors (white for 10's, green for 20's etc.). Vinegar (1975) used a variety of color combinations of model airplane paint to mark the tails of *Sceloporus undulatus*. She avoided using an excessive amount of paint in order to reduce any impact the paint might have on survivorship.

Stebbins and Cohen (1973) used a purple indelible pencil to color the base of the hind leg in *Sceloporus occidentalis* and thus indicate whether individuals had been parietectomized (left leg) or sham-operated (right leg). Similarly, Henderson (1974) used a felt-tip pen to write black numbers on the sides of *Iguana iguana* and reported that these marks lasted several weeks.

It is unknown how colored marks affect the behavior of congeners toward marked specimens in species for which body colors have seasonal social significance.

TAGGING

Minnich and Shoemaker (1970) marked *Dipsosaurus dorsalis* with colored Mystik cloth tape in addition to toe clipping. Different arrangements of the various colors of tape were placed around the base of the tail. This technique has also been used to mark *Uma scoparia* (Minnich and Shoemaker, 1972).

Uma notata have been tagged with a small piece of foil attached to a 30 cm, light string which was tied around the lower abdomen (Deavers, 1972). This tag allowed the measurements of burial depth of the lizards at night. Judd (1975) used a similar tag to locate buried *Holbrookia propinqua* for body temperature readings. He used a 5 cm square piece of aluminum foil and a 1 m length of red thread.

Rao and Rajabai (1972) tagged agamid lizards (*Sitana ponticeriana* and *Calotes nemoricola*) with different shaped, colored aluminum rings. These rings were placed around the thigh and caused no apparent hinderance. Henderson (1974) tagged *Iguana iguana* by tying small "jingle bells" around their necks with fishing line!

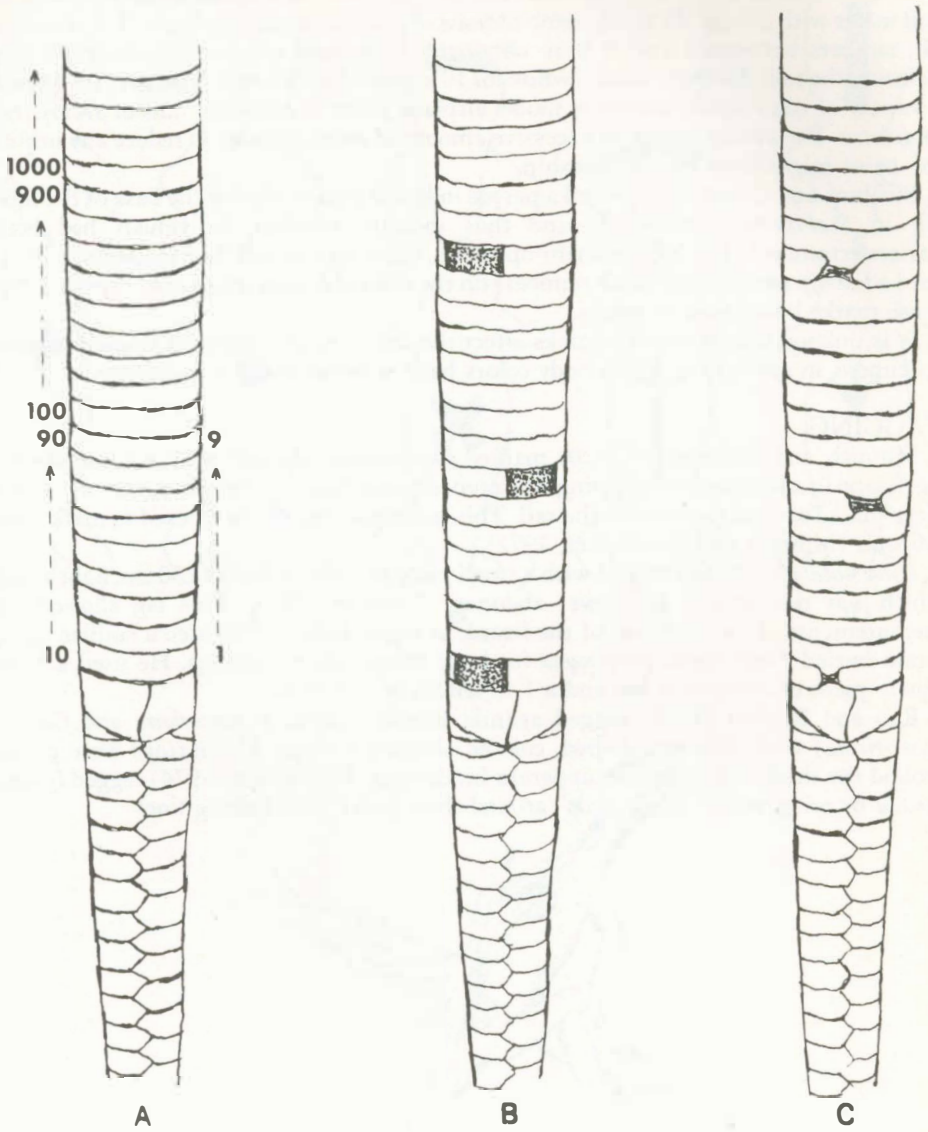


Figure 7. The ventral scale clipping system of Brown and Parker (1976b).

- A. The enumeration system.
- B. Freshly clipped snake No. 718.
- C. Scars on the same individual (No. 718) three years after marking.

Snakes

SCALE CLIPPING

Scale clipping with scissors or toenail clippers is by far the most commonly used method of marking snakes. Many investigators still follow the technique developed by Blanchard and Finster in 1933. They simply cut pieces from the subcaudals which would leave "permanent" scars. However, regeneration would be almost complete if the piece removed nearly the entire scale and integument down to the muscle layer with sharp-pointed scissors. They reported no adverse effects on the snakes.

Blanchard and Finster (1933) numbered the subcaudals on each side beginning at the proximal end of the tail. Therefore, if the fourth and sixth left and fourth right subcaudals are clipped, the individual was coded 4, 6-4. They usually scarred three scales per snake and only up to scale number 20. Since some snakes have a small postanal scale which could cause confusion as to which is numbered one, the most proximal subcaudal clipped on either side was always an even number.

Carlstrom and Edelstam (1946), working with *Natrix natrix*, criticized the technique of Blanchard and Finster (1933) in that regeneration was a problem and that it was difficult to mark hatchlings. Woodbury (1956) cites regeneration of clipped scales in 4 or 5 years as reported by Conant for *Elaphe obsoleta*. Weary (1969) found three disadvantages to the use of Blanchard and Finster's technique in marking *Storeria occipitomaculata* and *Thamnophis sirtalis*: 1) Several minutes were required to remove carefully a complete caudal scale; 2) Despite precaution, blood was frequently drawn, allowing possible infection; and, 3) Clipping proved to be very difficult on young snakes less than 10 cm long.

Brown and Parker (1976b) have described a ventral scale clipping system, which they feel fulfills more of the criteria for an ideal mark (see Introduction) than does Blanchard and Finster's (1933) method. They see it as an important improvement because ventrals are "larger and easier to clip than subcaudals" and scars in that area cannot be lost by tail breakage. Brown and Parker have also devised a serial enumeration system (see Fig. 7) which yields a whole number for each specimen and is "far less confusing" than the number code of Blanchard and Finster.

Brown and Parker (1976b) used sharp-pointed scissors of appropriate size and quality and described the procedure as follows:

- (1) Insert a tip of the scissors under the posterior edge of the scute to be clipped, push it forward beneath the width of the entire scute, and cut. Make two such incisions, one on each side of the intended block to be excised.
- (2) Insert the scissors under and across the top (anterior edge) of the scute and make a third cut transversely to remove the entire section. Depth of the cuts is important: the entire excision should include all layers of the skin (including the dermis) to expose the underlying ventral

musculature. Muscles should be visible and the section removed should be large enough to cover half of a given ventral. In cases of dual numerals involving the same scute (55, 66, etc.), clip all the way across and leave some lateral traces of the scute so that its position can be counted after healing. There is an apparent tendency for adjacent scutes to "invade" an excised area (see Fig. 7) or to adpress against an excision zone after removal of an entire scute. Numbers involving adjacent scutes on the same side (e.g., 190's, 1900's) should be omitted.

The authors did not observe direct adverse effects of this technique on snakes. However, they did relate scale clipping and/or handling as causes for increased over-winter mortality and weight loss in newly-marked *Masticophis taeniatus*. Their studies found no similar affects with *Coluber constrictor* or *Pituophis melanoleucus*. Brown and Parker reported these marks permanent for at least four years and often (92% of the time) shed skin from clipped *Coluber* would be precisely identified.

BRANDING AND PAINTING

Woodbury (1956) reviewed his tattooing technique for snakes which used a portable, battery powered tattoo outfit. Numbers were made on any light areas where pigmentation would not obscure any portion of the mark. In order to minimize misreading numbers upon recapture, the free ends of the numerals 2, 3, 4, 5, 6, and 9 were "made extra long" so that they were better distinguished from each other and from the numeral 8, having no free ends. Woodbury found this mark permanent and with no adverse effects after the recovery of the snake from the tattooing operation.

Storeria occipitomaculata and *Thamnophis sirtalis* have been branded with a 50 watt pyrographic needle which can be purchased in hobby shops (Weary, 1969). The needle was filed to give a sharp cutting edge and applied briefly to burn through scales without causing bleeding. No regeneration was observed over a two year period. If no house current is available, Weary suggested using the more expensive American Beauty soldering pencil, model B2000-L, powered by a 12 v rechargeable battery.

Clark (1971) branded several species of snakes using Hoskings Chromel "A" resistance wire (The Malin Co., Cleveland, Ohio) which is 20% chromium and 80% nickel. A 20 mm piece of wire (B and S gauge No. 24) fixed in a handle was used and heated with either a Bunsen burner or a small, portable propane torch. Brands were placed on the ventrals and/or subcaudal scutes using a numbering system similar to that of Blanchard and Finster (1933) which is described above. The obliteration of the brands by regeneration was reduced by including the first row of adjacent dorsal scales in the mark. This resulted in a healed area being covered by either a single scale "as though the ventral (or caudal) and adjacent dorsal had fused" or the normal division between the two scales was out of place. In addition, the regenerated portion may have a change in pigmentation.

The wound produced by Clark's (1971) brand forms a scab within a few days and is not an open sore. Sometimes, scales may be sloughed off before complete recovery by the underlying integument, leaving an open sore. Even in these cases, Clark believed survivorship to be less influenced by branding than by scale clipping. He also commented that the increased survivorship of branding over clipping may offset the inconvenience of having added equipment in the field. Clark had data suggesting that healing of the brands occurred more rapidly and with fewer complications in the field than when snakes were confined in the laboratory.

Lewke and Stroud (1974) marked two *Crotalus viridis* and seven *Pituophis melanoleucus* by freeze branding. A super-chilled, copper branding instrument was used to alter the normal pigmentation by destroying the chromatophores, but not permanently changing any other part of the integument. Bar and angle shaped irons (0.5 cm by 1.5 cm by 1.0 cm deep) were fashioned from a copper bar or tubing and soldered to

sturdy wire handles which were 15 cm long. The authors experimented with three coolants:

- (1) An equal part crushed dry ice - 95% ethyl alcohol slurry (-70° C).
- (2) Liquid freon 12 (boiling point, -21° C).
- (3) Liquid freon 22 (boiling point, -41° C). Both freons obtained from Virginia Chemicals, Inc., Portsmouth, VA in 500 g pressurized cans.

The coolants were placed in insulated containers and the irons were cooled until boiling stopped at the iron surface, "indicating that the metal had reached the temperature of the coolant." The brand site was swabbed with 95% ethyl alcohol to increase conduction and the branding iron was held against the integument for 5 to 30 seconds. Only the dry ice-alcohol coolant produced successful marks which were best at 20 to 30 second exposures. These marks have been observed for about two years and produced no adverse effects in the snakes. The brands may induce molting as all the snakes used in this study molted within three weeks of marking.

Both types of freon were used successfully by Lewke and Stroud (1974) when applied for 5 to 20 seconds with L- or bar-shaped synthetic foam sponges fastened to wooden dowling with a silicon adhesive. Applying the freon 22 by direct spraying over a styrofoam stencil for 1 to 2 seconds produced visible brands, but the shapes were usually distorted because of leakage around the stencil. The freon coolants were used primarily because they are more convenient to carry in the field.

The primary disadvantage of freeze branding, according to Lewke and Stroud, is that the mark is not visible until the first molt after branding. Additionally, the color background of the snake must be considered and a minimum branding iron size would be reached "because the heat sink available for cooling is a function of the mass."

R. K. Farrell (personal communication) has used a ruby laser to permanently mark king snakes and rattlesnakes. This work was done as a peripheral study to projects which concentrated on the marking of crabs and fish. This technique was developed for the rapid marking of extremely large numbers of individuals.

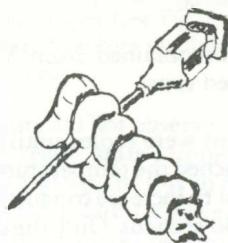
Pough (1966) marked three species of *Crotalus* "by painting an identifying number on the basal rattle segment with quick-drying waterproof paint (Testor's Butyrate Dope)." *Masticophis taeniatus* have been painted on the head and neck with a color code for individual recognition (Parker, 1976; Bennion and Parker, 1976).

TAGGING

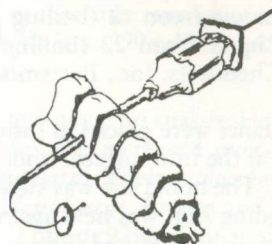
Hirth (1966) tagged adult *Masticophis taeniatus* and *Coluber constrictor* with numbered monel tags which were "clamped into the corner of the mouth." No details concerning this technique were given and it apparently has not been widely used by other investigators.

The Buttoneer (as discussed in the section on turtles) has been used to mark snakes by inserting a plug into the caudal musculature through the lateral region of a subcaudal scute (Pough, 1970). Plugs should be positioned so that the bulb rests tightly against the snake's integument. Difficulty may be encountered in marking smaller snakes (25 cm) because of their thin tails, but using a Buttoneer with a shortened tip may alleviate this somewhat.

Pendlebury (1972) tagged *Crotalus viridis* with a pair of colored vinyl discs which were fastened "through the dorsal lobe of the second proximal segment of the rattle" (see Fig. 8). The discs were 6.0 mm in diameter and 0.5 mm thick and were punched from large sheets (available at most plastic supply houses) with a paper punch. A 50 mm length of monofilament nylon fishing line was passed through a hole which had been made with a needle in the center of the pair of discs. One end of the line was melted into a bead and the other end knotted to secure the discs. Upon capture of the rattlesnakes, a No. 21



A



B



C



D

Figure 8. Illustration of the technique from Pendlebury (1972) for tagging rattlesnakes. See text for details.

hypodermic needle was passed through the rattle as illustrated in Fig. 8A. The knotted end of the line was then cut and one disc removed. This end of the line was then threaded through the needle, the needle was removed and the disc was replaced over the loose end of line (Fig. 8B and C). The cut end of the line is then beaded with a match and the snake may be released. Pendlebury reported that these tags lasted at least 15 weeks and believed them to be essentially permanent in that the more distal segments of the rattle are more subject to wear. Young rattlesnakes might be tagged on the "button" which would run more risk of loss. The tag was found to be readily observable from distances of 6.5 to 30 m (with binoculars). Using 10 colors of plastic discs, 100 individuals could be distinctively tagged. The number of molts which have occurred since tagging can be determined by $(N-1)$, where N is the number of segments craniad to the tagged one. Pendlebury reported no adverse effects of this technique.

INTEGUMENT PATTERNS

Carlstrom and Edelstam (1946) photographed the ventral, black and white pattern of *Natrix natrix*. They found this pattern to be of infinite variation, but constant during the life history of each individual. Therefore, they described this system of individual recognition to be "as sure as the fingerprints of the police."

Crocodylians

Chabreck (1965) reported on several methods which he used in marking *Alligator mississippiensis*. Toe clipping was successful and permanent, but was somewhat limited for use in individual recognition. So, toe clipping was combined with the notching of tail scutes (the ridged scales located dorsally and caudally on the tail). This method provided combinations for over 3000 distinct marks by assigning each scute a letter and each toe a number. In addition, a monel tag was attached to a dorsal tail scute in order to have a mark which was obvious to people not familiar with the study. This tag was of the self-piercing type, size no. 681 from the National Tag and Band Co. (Newport, Kentucky 41071) and was imprinted with a number and return address.

Additional techniques were tested by Chabreck and found to be less satisfactory for long term use: streamer tags, tattooing, and vinyl plastic collars. Joanen and McNease (1973) have used telemetry to tag alligators. Stephen J. Gorzula (personal communication) has successfully marked *Caiman crocodylus* by clipping triangular pieces out of the ventral tail scutes according to a coding system. He has found the marks identifiable for two years, but has generally remarked individuals after one year as regeneration of the scales does make them more difficult to read after that time.

Radioactive Tagging

Radioactive tagging may be a particularly valuable technique for tracking organisms that are too small for a transmitter and which need to be followed for long periods of time, totally undisturbed. This technique has been increasingly used in marking herpetofauna as is reviewed below. Special attention needs to be given to licensing procedures for the field use of isotopes. Much of the information on licensing and the use of radioisotope tags with salamanders has been provided by Ray E. Ashton, Jr.

LICENSING

Licensing procedures for radioisotope use are handled through appropriate regulatory agencies of the state, not through the Atomic Energy Commission. In Kentucky, for example, this agency is the Radiation Control Branch of the Bureau for Health Services in the Department for Human Resources. The applications ask two major questions. The first is the experience of the investigator, including courses in safety and practical experience. Formal training in radiation safety procedures is offered on most major university campuses. None of these courses relates to the field use of radioactive isotopes, however. Therefore, one must receive training from someone experienced in the use of field tagging techniques. This training is not only valuable in learning the technique, but can also be cited as experience on the licensing application.

The second major area of concern is the project itself and its location. It is very important to make every attempt to explain how there will be a very little chance of accidental exposure to radiation by the public. The more explicit the information you provide on step by step procedures to be utilized when tagging, monitoring and recovering the specimen to be studied, the more rapid the final action on your application will occur. Selection of the isotope must be made carefully; the tags should not be too large or powerful. Even though a long half-life is better for ideal field work, this factor may be strictly limited by the review board of the regulatory agency. For example, Henry Fitch (personal communication) has received clearance for the use of Tantalum 182 (half-life of about 160 days) in some studies, but not for Cobalt 60 (half-life of 5.8 years). In many other locations Cobalt 60 has received clearance. At this time, I am not aware of specific guidelines (state or federal) for the use of radioactive materials as tags for marking animals in field studies.

Most often, researchers on university campuses must go through a sub-permit procedure. This is done through the appropriate department that is in charge of radioactive safety responsibilities for the institution. The permit granted is for the purchase and use of isotope tags, and is usually very specific as to storage facilities, number of tags and location of use.

TECHNIQUES

The following review of techniques used for various amphibians and reptiles contains

information for the selection of isotopes, application of tags and use of detection equipment.

Ambystoma larvae have been marked for short periods with ^{24}Na with no observed increase in mortality (Shoop, 1971). Shoop treated the larvae in the field for 90 minutes in a 500 ml solution of 10 microcuries $^{24}\text{NaCl}$ (10 ml NaCl solution of varying salt concentrations) and pond water. Larvae were then rinsed in two changes of water, released and recaptured the following day. Individual larvae which had been tagged with ^{24}Na were detected with a portable, crystal scintillation spectrometer. Due to the short half-life of ^{24}Na , tagged individuals are not recognizable after three to six days.

Karlstrom's (1957) study of *Bufo canorus*, the Yosemite toad, is the first major discussion of the use of radioactive tagging (^{60}Co) for amphibians. Another commonly cited study with anurans is that of Breckenridge and Tester (1961). They tagged *Bufo hemiophrys* with ^{182}Ta wire which was inserted beneath the skin with a large, modified hypodermic syringe.

Barbour, *et al.* (1969a) tagged *Desmognathus fuscus* with a 0.75×3.3 mm piece of $45\mu\text{C}$ ^{60}Co alloy wire. These wires were placed in the tail musculature with a modified hypodermic needle. No adverse effects were observed. A detection apparatus (Thyac II Model 489 survey meter) could locate tagged individuals at a distance of from 3 to 4 m when they were at or near the surface. *Plethodon jordani* have been tagged with similar injections of 18 gauge, $20\text{--}48\mu\text{C}$, ^{182}Ta wire (3 to 5 mm long) into the abdomen (Madison and Shoop, 1970).

Ashton (1975) used ^{60}Co (35–50 μC) alloy wire (ICW Pharmaceutical, C & R Division, 2727 Campus Dr., Irvine, CA 92715), about 3.0×0.5 mm in size to tag *Desmognathus fuscus*. The tag was inserted as described by Barbour, *et al.* (1969a) and monitored with a Victoreen Thyac III Model 491 survey meter with a scintillation probe. In this study and others with plethodontids, Ashton has observed local ulceration which eventually opened, exposing the tag. Ashton reports that this "burn out" has not been a problem with *Pseudacris*, *Necturus* or in reptiles. He also reports that platinum coated wires are excellent for reducing local tissue damage in smaller animals and that sterile procedures are important to avoid infection.

Three species of aquatic turtles (*Kinosternon subrubrum*, *Pseudemys scripta*, and *Deirochelys reticularia*) were tagged with radioactive pins of ^{182}Ta (100 μC , 1×5 mm) which were placed in each of two, 2×6 mm holes drilled in the upper portion of the bridge (Bennett, *et al.*, 1970). The pins were held in place with waterproof cement (Dupont "duco"). The turtles were relocated with a portable, beta-gamma meter (EP 432) with a maximum range of 7 m when used in conjunction with NaI-Tl activated crystals. Similarly, Ward, *et al.* (1976) marked *Clemmys guttata* with one radioactive pin (^{182}Ta , 100 μC , 1×5 mm) in the 10th or 11th marginal scute.

O'Brien, *et al.* (1965) tagged one *Sceloporus undulatus hyacinthinus* externally with gold 198 which was placed in polyethylene tubing and tied around the waist of the lizard. The tag was detectable from distances of 5 to 12 feet for about one week and then recapture was necessary to attach a new tag. In addition, Henry Fitch (personal communication) has successfully used ^{182}Ta wires (0.8×2 mm, 100 μC) on *Eumeces fasciatus*, *Scincella laterale*, *Ophisaurus attenuatus*, *Diadophis punctatus* and *Carphophis amoenus*. This tag was not used successfully with *Storeria dekayi* because it is more mobile than the others and is soon lost in the field. He can detect the tags from about 30 feet under field conditions and so recommends use of this tag only for more sedentary animals. Barbour, *et al.* (1969b) studied *Carphophis amoenus* with ^{60}Co tags. Inglis, *et al.* (1968) described a device for automatic detection of small animals carrying radioactive tags which may have application for herpetological studies.

Radiotelemetry

The use of radiotransmitters as a marking device may be applicable to certain studies. Often these devices are also used to monitor and transmit physiological parameters (temperature, heart rate, etc.). As a marking technique, radiotransmission offers such advantages as providing a constant record of the animal's location (day or night, above or below substrate) and allowing the investigator to remain some distance from the animal and, therefore, have a minimal influence on its behavior. Disadvantages of this technique include its relative complexity, cost, feasibility depending on the size of the animal, and limitations of the power source. In this review, I will attempt to indicate how radiotelemetry has been used with reptiles and hopefully give enough information so that you can determine if this technique is appropriate for your study. Due to the complexity of the technique, however, an investigator is expected to consult other sources in adapting this technique to a specific problem.

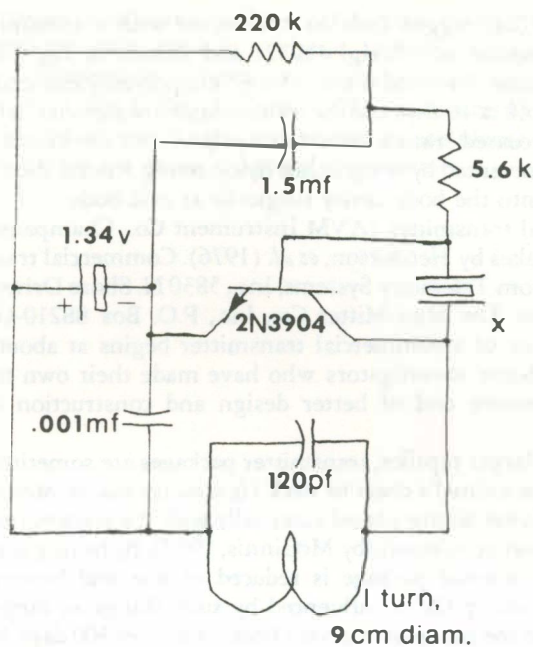
THE TRANSMITTER

Many investigators construct their own transmitters or choose from a few that are now commercially available. If you use radiotransmission and have a limited background in electronics, whether or not you construct your own system, reading the chapter on electronics in Mackay (1970) is a must. Mackay also has basic information on the construction of various transmitters. The unit used by Shirer and Downhower (1968) is illustrated in Fig. 9A and has been adapted for use with several reptiles (*Elaphe*, *Pituophis*, *Coluber*, *Agkistrodon*, *Lampropeltis*, *Thamnophis*, *Natrix* and *Crotalus* by Fitch and Shirer, 1971; *Trionyx* by Plummer and Shirer, 1975; *Terrapene* and *Chelydra* by Fitch (personal communication)). The transmitters used by Fitch and Shirer (1971) were described as follows:

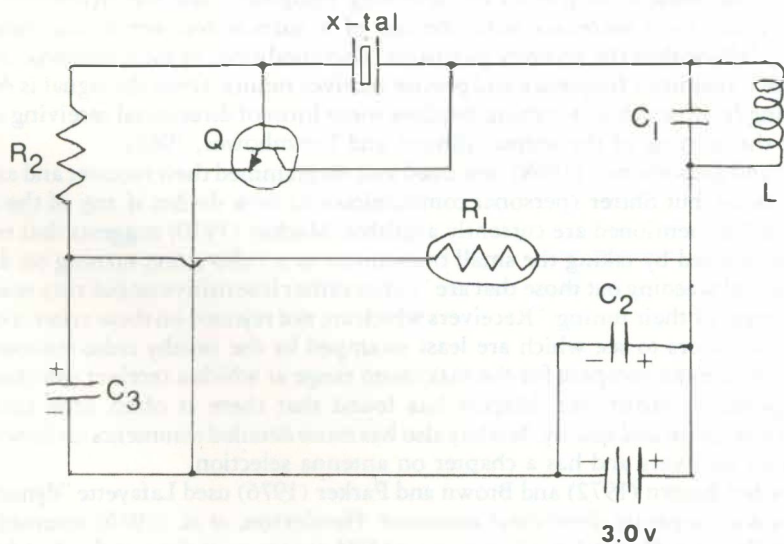
The transmitters were self-pulsed, crystal controlled transistor oscillators operating at 5 kHz intervals between 26.600 and 26.750 MHz. One to 2 ma collector current pulses of 50-100 msec duration and repetition rates of 1-2/sec, along with a 250 ma-hr mercury battery, yielded an average life of one month. The radiating collector tank inductance was wound around the periphery of the unit on an elliptical balsa form about 45 x 18 mm. The size of the inductance was a compromise, with an area sufficiently large for effective radiation but yet sufficiently small for instrument ingestion by the snake. After assembly of all components except the battery, the unit was vacuum impregnated with epoxy. The battery was inserted in a close fitting hole within the form and after soldering in place the units were dipped in beeswax for moisture sealing. A final dip coating of silicone rubber produced a very smooth, oval package about 50 x 21 x 14 mm weighing about 16 g. Immediately before emplacement, field strengths (about 500 v/m at 1 m) and frequencies were checked.

Parker and Brown (1972) used long range transmitters, similar to those used by Osgood (1970) for *Natrix*, to tag *Masticophis taeniatus* in a study of movements. Brown

- Figure 9. A. Circuit diagram of the transmitter from Shirer and Downhower (1968). X is a third overtone crystal between 26.500 and 26.650 MHz.
- B. Schematic diagram of the tracking transmitter from Osgood and Weigl (1972). Component values: L — 4 turns, #24 enameled magnet wire 2-3 cm (diameter to suit animal); Q — G. E. D26 E-5; C¹ — to match L, 50 pfd; C² — 0.01 mfd tantalum; C³ — 1-6.8 mfd tantalum (value selected to produce desired click rate); R¹ — 1 Megohm bead thermistor; R² — 1 K $\frac{1}{8}$ watt; X-tal — miniature walkie-talkie transmit crystal of desired frequency; batteries — 2 Mallory MS-13 silver oxide in series. The components were assembled alongside the crystal can and coated with epoxy to both seal and anchor them in place.



A



B

and Parker (1976a) tagged *Coluber constrictor* with a transmitter similar to the one described by Osgood and Weigl (1972) and shown in Fig. 9B. Brown and Parker's transmitter weighed 11 g and was a 1.3 x 3.5 cm polyethylene-encapsulated cylinder. The battery life was 40 ± 10 days and the transmission frequencies in the range of 26.87-26.94 MHz. Paraffin-coated transmitters were palpated into the stomachs of some snakes and regurgitation prevented by tying a thin nylon string around the body. Other transmitters were inserted into the body cavity surgically at mid-body.

A commercial transmitter (AVM Instrument Co., Champaign, Illinois) was adapted for use with snakes by Henderson, *et al.* (1976). Commercial transmitters for snakes are also available from Telemetry Systems, Inc., 5830 N. Shore Drive, Milwaukee, Wisconsin 53217, and from The Mini-Mitter Co., Inc., P.O. Box 88210-G, Indianapolis, Indiana 46208. The price of a commercial transmitter begins at about \$55.00, depending on specifications. Some investigators who have made their own transmitters have found them less expensive and of better design and construction than some commercial equipment.

In studies of larger reptiles, transmitter packages are sometimes placed on a sling and hung around the animal's chest or neck (*Iguana iguana* by Montgomery, *et al.*, 1973) or may have the power source placed externally with the transmitter implanted in the body cavity (*Sceloporus occidentalis* by McGinnis, 1967). By having the power source mounted externally, the internal package is reduced in size and battery replacement is made possible. The battery life is influenced by such things as range and pulse rate of the transmitter, and the life span may vary from 14 to over 300 days. Small, solar transmitters (Telemetry Systems, Inc.) are available which would require no power supply replacement for external packages.

THE RECEIVER AND ANTENNA

The means of receiving and converting the transmitted signals must be precisely selected. The system should be capable of detecting very weak signals which is somewhat limited by the noise arising from the receiving equipment and the environment. The signal-to-noise ratio increases with the use of a narrow receiver if the transmitter frequency falls within the receiver passband. This condition, in turn, demands a known and stable transmitter frequency and precise receiver tuning. Once the signal is detected, the bearing from which it is coming requires some form of directional receiving antenna to allow the locating of the animal (Shirer and Downhower, 1968).

Shirer and Downhower (1968) described and diagrammed their receiver and antennae in great detail, but Shirer (personal communication) now doubts if any of the specific subassemblies mentioned are currently available. Mackay (1970) suggests that receivers might be selected by taking the small transmitter to a radio store, turning on different receivers, and weeding out those that are "either rather insensitive or not very selective in the sharpness of their tuning." Receivers which are not rejected on these criteria can then be tested outdoors to see which are least swamped by the nearby radio stations. From these, then, one can compare for the maximum range at which a receiver can clearly pick up the portable transmitter. Mackay has found that there is often little correlation between size, price and quality. Mackay also has more detailed comments on how to select and modify receivers and has a chapter on antenna selection.

Parker and Brown (1972) and Brown and Parker (1976) used Lafayette "dynacom-12" receivers with separate directional antennae. Henderson, *et al.* (1976) received signals directionally on a Model LA 12 receiver (AVM Instrument Co.) and a hand held, 3-element yagi antenna. Commercial receivers (walkie-talkies) were also used by Osgood (1970). A Sony CB107W Citizen Band transceiver with slight modification was used by Stebbins and Barwick (1968), with tuned direction-finding loop antennas (11 inch diameter of heavy coaxial cable).

Shirer (personal communication) has found reception much more of a problem than transmission, and the cost per unit certainly is higher. Direction finding receivers are becoming commercially available and may be quite applicable to radiotelemetry. One such receiver is the Portable DF, Little L-Per (L-Tronics, 5546 Cathedral Oaks Road, Santa Barbara, California 93111) which seems ideal except that its usefulness depends on the pulse width being greater than are many in animal transmitters.

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